ABSTRACT

A method for measuring free ligands in biological fluids in the presence of bound ligand and endogenous binding proteins, without disturbing the equilibrium between the free ligand and the protein-bound ligand, comprised of the following steps: (a) incubating a sample of biological fluid with (i) a ligand analog tracer which, due to its chemical structure, does not bind to some of the endogenous binding proteins, (ii) a specific ligand binder and (iii) specific chemical inhibitor reagents that alone or in combination inhibit the binding of the ligand analog tracer to other endogenous binding proteins; (b) separating the ligand analog tracer bound to the specific binder from unbound tracer; and (c) comparing the bound fraction in said sample to the bound fraction of a given set of known free ligand calibrators to determine the concentration of free ligand in said biological fluid.